Zuccagnia-type Propolis from Argentina: A potential functional ingredient in food to pathologies associated to metabolic syndrome and oxidative stress

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Abstract: The effect of Argentine propolis extracts against enzymes related to metabolic syndrome and oxidative stress, as well as the botanical origin of raw propolis, were studied. Histological and chemical analyses of propolis samples revealed that the botanical origin is *Zuccagnia punctata*, an Argentine medicinal plant. The melissopalynological analysis showed both pollen grains of *Z. punctata* and the other plant species. This result indicates that the differences found in the botanical remains compared to the palynological studies may have been caused by the bees selecting resinous shrubs mainly of *Z. punctata* for the production of propolis and other plants with flowers for the production of honey.
The richness of propolis was remarkable in two flavonoid precursors (2′,4′-dihydroxy-3′-methoxychalcone, 2′,4′-dihydroxychalcone), the major chemical components of *Z. punctata*. The hydroalcoholic extracts of Argentine propolis and *Z. punctata* were able to inhibit enzymes associated with the metabolic syndrome, including α-glucosidase, α-amylase and lipase, with IC_{50} values between 7 to 14, 37 to 48, and 13 to 28 μg/mL, respectively. Biological activity was mainly attributed to chalcones. Oxygen and nitrogen reactive species scavenging activity was determined by the assays of superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($HO^{\bullet}$), hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\bullet}$), and cation radical ($ABTS^{\bullet +}$). Results showed SC_{50} values between 115 to 278, 12.50 to 46; 39 to 92; 50 to 104.50 and 23 to 33.75 μg/mL, respectively. This study suggests for the first time that propolis from Argentina is highly effective in inhibiting enzymes related to the metabolic syndrome and in free-radical scavenging that would justify its use as a dietary supplement or as a functional ingredient in special food.

Keywords: Argentine propolis, chalcones, free-radical scavenging activity, metabolic syndrome, *Zuccagnia punctata*

Practical Application: Propolis from Catamarca, Argentina, is traditionally used as medicine and food. Its botanical origin is *Zuccagnia punctata*, an endemic plant species popularly used as a medicine in Argentina. Propolis has the ability to regulate the activity of enzymes involved in the carbohydrate and lipid metabolism, and consequently in metabolic syndrome. Besides, its antioxidant capacity makes it a natural product that can be used as a dietary supplement or as a functional ingredient in special foods. It is important to highlight that in the Argentine Food Code, propolis was incorporated in 2008 as a dietary supplement and the present results give major added value to this product.

1. INTRODUCTION

Metabolic syndrome is a disorder associated to several pathologies: mainly diabetes, obesity, and cardiovascular condition. Slowing the digestion and breaking down starch may have beneficial effects to insulin resistance control. For this reason, both inhibition of enzymes related to carbohydrate metabolism, that is, α-glucosidase and α-amylase, and inhibition of lipolytic enzymes, such as lipase, are the most common therapeutic approaches to treat metabolic syndrome (Costamagna et al., 2016; Herrera, del Hierro, Fornari, Reglero, & Martin, 2019; Spínola & Castillo 2017). Thus, the discovery of hypoglycemic and hypolipemiant natural agents with a strong power and a weak side effect is highly desirable.

Propolis is a natural product obtained by *Apis mellifera* from resins collected from plants, widely used due to its medicinal properties (Coelho, Falcao, Vale, Almeida-Muradian, & Vilas-Boas, 2017). In Argentina, propolis was included in the food code as an ingredient in sweets, honey, ethanolic extracts, and dietary supplements (Argentine Food Code, Chapter XVIII). Different propolis types have been reported in Argentina, depending on their botanical and geographical origin. *Larrea divaricata* and exotic *Populus* species are source of propolis from the Andean region (Isla et al., 2009; Kumazawa, Ahn, Fujimoto, & Kato, 2010). *Zuccagnia*

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Table 1–Botanical studies of propolis samples collected from Catamarca.

Sample	Department	Locality	masl	Coordinates	Botanical species	Family	%*
C-I	Pomán	El Rincon	1595	28°13'S 66°08'O	*Z. punctata Larrea sp. Sp1*	Fabaceae Zigophyllaceae Nonidentified	100 20 5
C-II	Santa María	Santa María	1885	26°41'S 66°02'O	*Z. punctata*	Fabaceae	100
C-III	Santa María	Santa María	1885	26°41'S 66°02'O	*Sp1 Z. punctata*	Nonidentified Fabaceae	100 100

*Percentage of samples with at least a fragment of a species.
masl, meters above sea level.

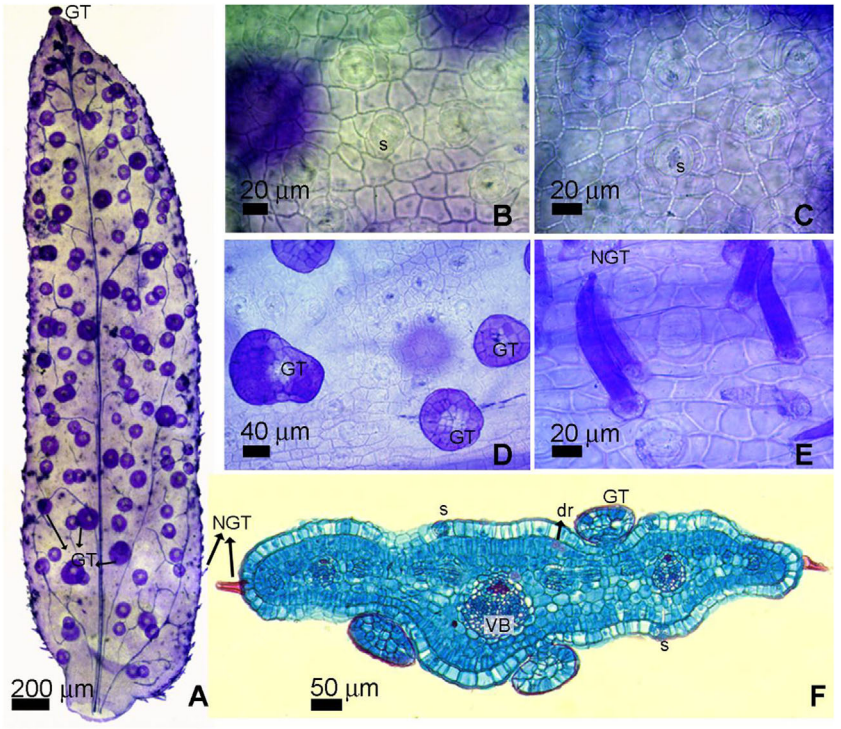

Figure 1–Plant material of *Zuccagnia punctata* (A) Leaflet aspect. (B) Upper epidermal cells, adaxial epidermis. (C) Abaxial epidermal cells. (D) Capitate glandular trichomes (GT). (E) Nonglandular trichomes (NGT). (F) Leaflet cross-section showing marginal nonglandular unicellular trichomes and capitate glandular trichomes in crypts.
References: dr, druse; GT, glandular trichome; NGT, nonglandular trichome; s, stoma; VB, vascular bundle.

punctata has been assigned as the main botanical constituent of the propolis from semiarid region of Tucumán, which has been characterized by the presence of chalcones (2′,4′-dihydroxy-3′-methoxychalcone, 2′,4′-dihydroxychalcone) as chemical markers (Salas, Alberto, Zampini, Cuello, et al., 2016; Salas, Mercado, Zampini, Ponessa, & Isla, 2016). Both the bioactive chemical components and the functional properties, such as antifungal, antibacterial, antioxidant, anti-inflammatory, and antimutagenic, were previously reported in some Argentinean propolis types (Agüero et al., 2010, 2011, 2014; Isla, Paredes Guzman, Nieva Moreno, Koo, & Park, 2005; Isla et al., 2009, 2012; Nieva Moreno, Isla, Cudmani, Vattuone, & Sampietro, 1999; Nieva Moreno, Isla, Vattuone & Sampietro, 2000; Nieva Moreno, Zampini, Ordoñez, Vattuone, & Isla, 2005; Salas et al., 2014, 2016; Salas, Zampini, Maldonado & Isla, 2018; Solórzano et al., 2012, 2017; Vera et al., 2011).

In this study, propolis samples were collected from Monte of Sierras and Bolsones, Catamarca, Argentina. This ecoregion shows a wide variety of characteristic native plant species such as jarillas, retama, mata sebo, montenegro, brea, pichana, and others (Morello, Matteucci, Rodriguez, & Silva, 2012).

The aim of this article was to analyze the effect of propolis from Catamarca, Argentina, on enzymes linked to carbohydrate and lipid metabolism, as well as on oxidative stress and its botanical origin.

2. MATERIALS AND METHODS

2.1 Propolis and plant species samples

Propolis samples were collected from apiaries of National Apiculture Program INTA-PROAPI during four successive years between 2015 and 2018 from beehives located in El Rincón (C-I; 28°13' S 66°08' O) and Santa María (C-II, C-III; 26°41' S 66°02' O), Catamarca, Argentina (Monte of Sierras and Bolsones ecoregion). The samples were collected following the usual method of scraping during the annual cleaning of the beehive. Grids were collected and frozen, and propolis were recovered, and stored at −80 °C for further use.

Aerial parts of *Zuccagnia puntacta* Cav.; *Larrea cuneifolia* Cav., and *L. divaricata* Cav. were collected at the site where the beehives were located. All the plants were identified by Soledad Cuello PhD (INBIOFIV-CONICET). Samples were dried in a forced air oven at 40 °C.

2.2 Preparation of propolis and native plant extracts

Samples of raw propolis, *Z. punctata*, *L. divaricata*, and *L. cuneifolia* (20 g) were extracted with 250 mL of ethanol:water 80:20 v/v assisted by ultrasound for 30 min. Propolis extracts (PE) were taken to dry under reduced pressure, lyophilized, and stored at −20 °C in the dark until analysis.

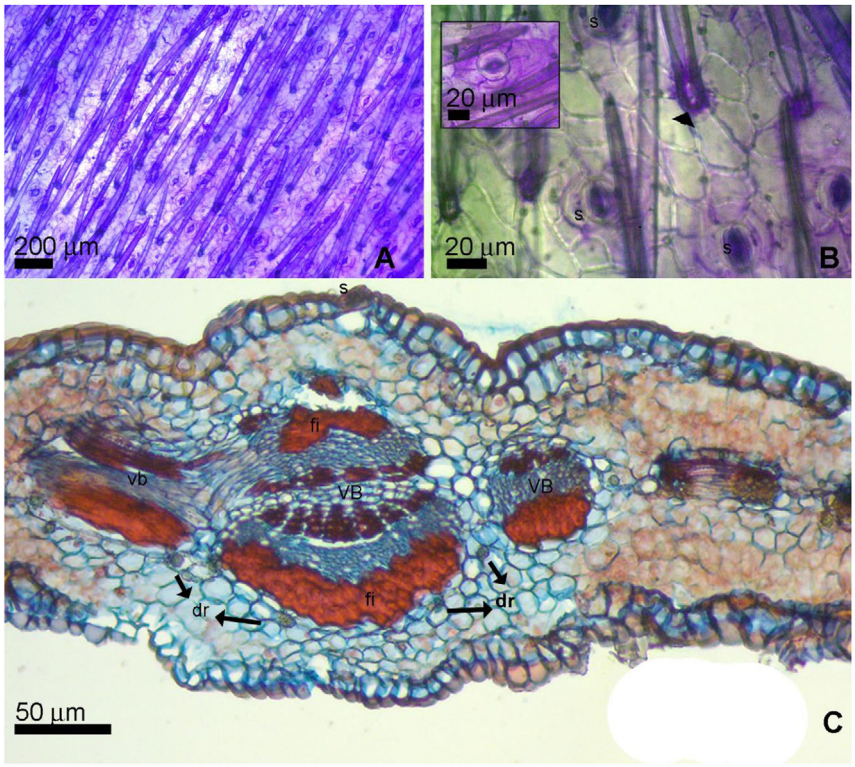

Figure 2–Plant material of *Larrea cuneifolia*. (A) Upper epidermis aspect with abundant nonglandular trichomes. (B) Lower epidermal cells, insertion of unicellular nonglandular trichome (arrow head), anomocytic stoma (s) and cyclocytic stoma (in insert). (C) Leaf cross-section. References: dr, druse; fi, fibers; s, stoma; VB, vascular bundle.

2.3 HPLC-DAD analysis

Phenolic compounds taken out from propolis and native plant species extracts were analyzed by the HPLC system (Water Corporation, Milford, MA) coupled to a diode array detector 2998 (220 to 540 nm). A C-18 column (Water X-bridge) and a solvent system consisting of 9% acetic acid in water (A) and methanol (B) as follows: 90 to 43% A over 45 min, followed by 43 to 0% A, from 45 to 60 min, and finally 0% A, from 60 to 65 min were used. Detection of chemical component was in UV at 330 nm. Empower ™ software was used. Compounds were identified by retention time and UV spectra and confirmed by comparison with standards. Three replicates were performed for each sample.

2.4 Fractionation of the PE

Both the fractionation of PE and the identification of the most active fractions were performed according to Salas, Alberto, Zampini, Cuello, et al. (2016). The PE was permeated on Sephadex LH-20 by using methanol as a mobile phase. Seven fractions were obtained. The fractions were analyzed by HPLC-DAD according to Salas, Alberto, Zampini, Cuello, et al. (2016).

2.5 Botanical analysis of propolis by using microscopic techniques

For botanical analysis, samples of each raw propolis (0.5 g) were processed according to Salas, Mercado, Zampini, Ponessa, and Isla (2016). Botanical identification was made by comparing observed structures with both plant materials of *Z. punctata*, *L. divaricata*, and *L. cuneifolia* collected at the site where the beehives were located and with relevant literature (Álvarez et al., 2012; Lersten & Curtis, 1996; Mercado et al., 2013, 2018; Moreno et al., 2015). Plant material was prepared according to Mercado et al. (2018) for conventional optic microscopy observations.

Observation of material was made under a Zeiss Axiolab optical microscope equipped with a Zeiss Axiocam ERc 5s digital camera and the measures were calculated by using the AxioVision Rel. 4.8 software.

2.6 Palynological analysis of propolis

The propolis were treated with KOH, heated, centrifuged, resuspended in distilled water and filtered (0.3 mm mesh filter) to remove large particles. Then, it was subjected to acetolysis (Erdtman, 1960). Samples were stained according to Wodehouse (1935). Pollen grain determination was carried out by comparison with reference collections of the Fundación Miguel Lillo (Palinoteca PAL-TUC) and/or using palynological Atlas (García, Reyes, & Rios, 2012).

Observations and quantitative analysis were carried out with a Zeiss Axiolab Lab.A1 optical microscope equipped with a Zeiss Axiocam ERc 5s digital camera and AxioVision Rel. 4.8 and Zen 2 (Blue Edition) 2.0. software. The pollen was classified as dominant (D), medium (M), lower (L), or trace (T) according to following percentage of >45%; 16 to 45%; 3 to 15%, and <3%, respectively.

2.7 Effect of propolis samples on enzymes of metabolic syndrome

2.7.1 Effect on α-glucosidase and α-amylase. Enzymatic inhibition assays were performed according to Costamagna et al. (2016). The preincubation of the α-glucosidase and α-amylase with different concentrations of PE (6 to 50 µg/mL) or positive (orlistat) and negative (solvent) controls was carried out at 4 °C for 10 min. To start the reaction, the substrate, that is, *p*-nitrophenyl-α-D-glucopyranoside or starch, respectively, was added. The reactions were incubated 15 min at 37 °C. Then,

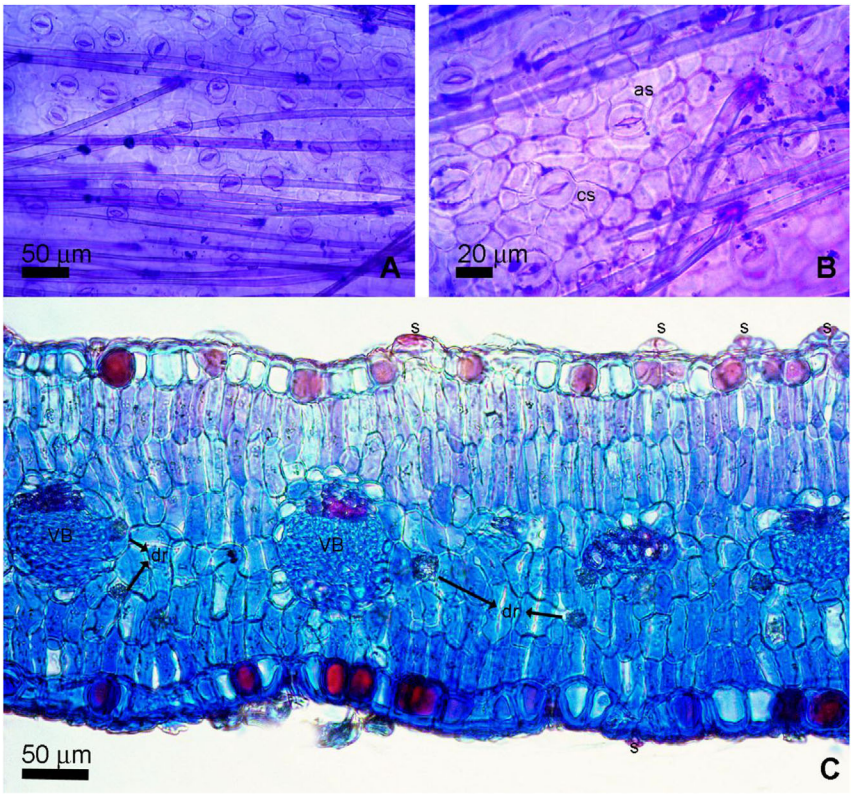

Figure 3–Plant material of *Larrea divaricata*. (A) Upper epidermis with abundant nonglandular trichomes. (B) Lower epidermis, with anomocytic (as) and cyclocytic stoma (cs). (C) Leaf cross-section. References: dr, druse; s, stoma; VB, vascular bundle.

sodium carbonate or iodine solution, respectively were added. The absorbance was measured at 405 nm to α-glucosidase assay or 640 nm to α-amylase assay. The results were expressed as IC_{50} values, that is, µg/mL of PE required to inhibit the enzyme activity by 50%.

2.7.2 Effect on lipase. The effect of PE on lipase activity was assayed according to Costamagna et al. (2016) by using final concentration of PE between 6 and 48 µg/mL, lipase and *p*-nitrophenylpalmitate as substrate. The amount of released *p*-nitrophenol at 37 °C was determined at 400 nm. IC_{50} values were determined as µg/mL of PE required to inhibit the enzyme activity by 50%.

2.8 Antioxidant activity of Argentinean propolis extracts

Spectrophotometric methods were used. The scavenging percentage of each free radical and H_2O_2 with and without PE was calculated. Scavenge percentage versus concentration is plotted and the SC_{50} values are determined as the propolis concentration (µg/mL) necessary to scavenge 50% of free radicals or H_2O_2.

2.8.1 ABTS scavenging activity. The effect of PE on free radicals was determined according to Orqueda et al. (2017) by using different concentrations of PE (10 to 50 µg/mL) in the presence of an $ABTS^{\bullet+}$ solution, with absorbance of 0.7 at 750 nm. The percentage of scavenging was measured spectrophotometrically at 750 nm after 6 min of incubation.

2.8.2 Hydroxyl and superoxide radical scavenging activity. The effect of PE on superoxide radicals was determined according to Valentao et al. (2001) by using NADH/phenazine methosulfate (PMS)/nitro blue tetrazolium chloride (NBT) system and different concentrations of PE (10 to 300 µg/mL). The reduction of NBT to the formazan blue chromogen by superoxide radicals was monitored spectrophotometrically at 560 nm.

Hydroxyl radicals scavenging capacity was determined according to Chobot, Hadacek, Bachmann, Weckwerth, and Kubicova (2016) by using the system 2-deoxy-D-ribose/$FeCl_3$/EDTA/H_2O_2/ascorbic acid with and without PE. The mixture was incubated at 37 °C for 60 min. Then, 2-thiobarbituric acid was added and heated at 100 °C for 20 min. The absorbance was measured at 532 nm.

2.8.3 Hydrogen peroxide scavenging activity. The H_2O_2 scavenging activity was measured spectrophotometrically at 504 nm (Orqueda et al., 2017) by using the system H_2O_2/phenol solution/4-aminoantipyrene/horseradish peroxidase with different concentrations of PE (20 to 100 µg/mL).

2.8.4 Nitric oxide radical scavenging activity. The PE capacity to NO scavenge was performed according to Govindarajan et al. (2003) by using a concentration range between 20 and 200 µg/mL. The assay mixture containing different concentrations of PE and sodium nitroprusside was incubated for 60 min at 37 °C. Then, Griess reagent was added and incubated for 15 min in the dark. The antioxidant capacity was measured spectrophotometrically at 550 nm.

2.9 Statistical analyses

The statistical *InfoStat* (Student Version, 2011) software was used.

3. RESULTS AND DISCUSSION

3.1 Botanical origin of propolis from Catamarca

The quality of propolis and its botanical origin were determined by microscopic analyses to botanical identification of fragments of leaves or other debris left (Salas, Mercado, Zampini, Ponessa, and Isla , 2016). In the present study, according to microscopical

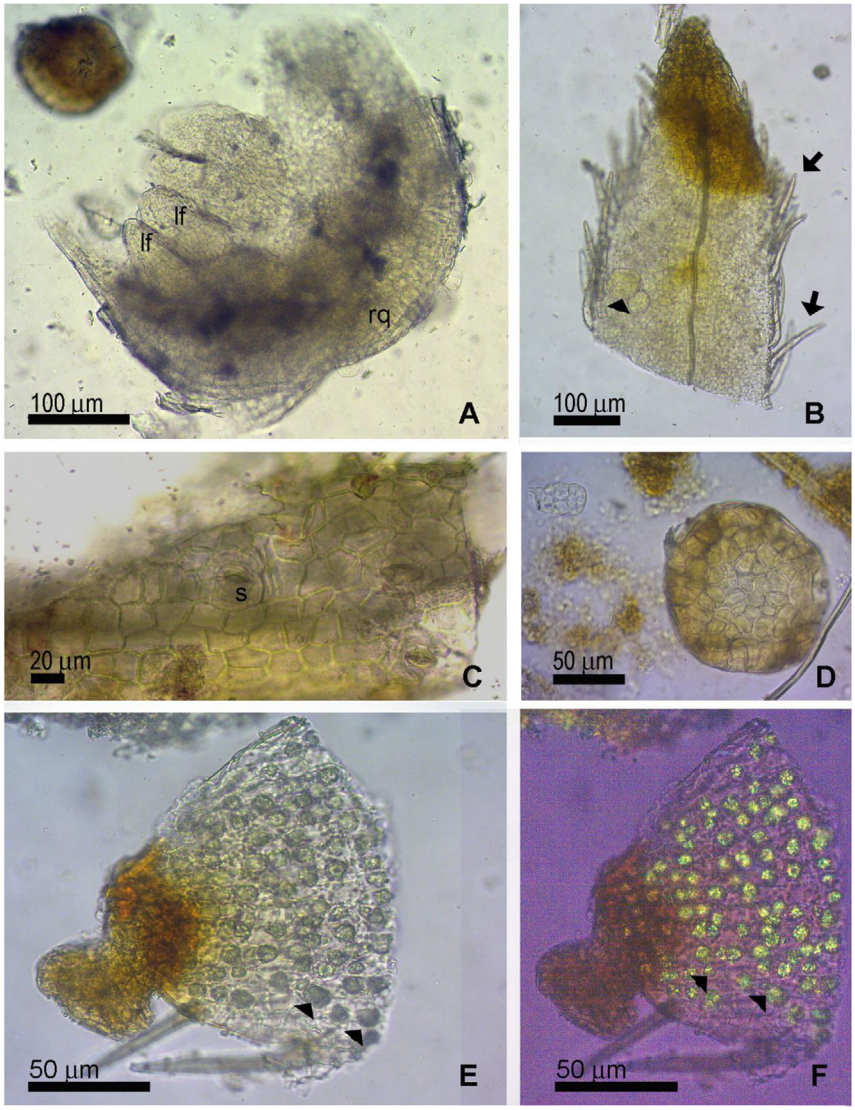

Figure 4–Botanical studies of propolis samples. Plant material identified as *Zuccagnia punctata* (Fabaceae). (A) Leaf primordia, with raquis (rq) and leaflets (lf). (B) Leaflet fragment with nonglandular unicellular trichomes (arrow) with a thick cuticle and a blunt apex and rounded multicellular glands (arrow head). (C) Epidermal cells with straight anticlinal walls and striated cuticle. Cyclocytic stoma (s). (D) Multicellular capitate glandular trichomes. (E-F) Druses observed under white and polarized light (arrow head).

analyses, propolis samples from Catamarca, Argentina showed the presence of *Z. punctata, Larrea* sp., and nonidentified species in different ratios (Table 1). In 100% of analyzed samples *Z. punctata* material was found (Table 1). *Zuccagnia punctata* and *Larrea* sp. were identified by comparison with plant samples. Aerial parts of *Z. punctata*, *L. cuneifolia*, and *L. divaricata* were analyzed by conventional microscopic techniques (Figures 1 to 3).

Microscopic analyses of propolis samples revealed the presence of *Z. punctata*, which was identified by the presence of compound leaf primordia with subopposite nanophyll leaflets (440.23 ± 167.87 mm long, 156.65 ± 57.21 mm lat) with acuminate apex, rounded, symmetrical base, and entire margin (Figure 4A), free leaflet fragments with spherical to oval sunken capitate multicellular glandular trichomes (163.75 ± 93.12 µm of diameter) (Figure 4B) and unicellular nonglandular trichomes (299.87 ± 10.73 and 45.21 ± 4.21 µm) (Figure 4B) arranged on the adaxial base of the foliar surface and on the foliar margins (368.44 and 44.21 µm), leaflet epidermal cells with straight anticlinal walls (Figure 4B and 4C), cyclocytic stoma, rarely paracytic or anomocytic on both epidermal surfaces (32.51 ± 2.15 µm long and 27.32 ± 2.58 µm lat) (Figure 4C) and druses in the mesophyll (Figure 4E and 4F). Isolated multicelullar glandular trichomes were also observed (Figure 4D). These features respond to those previously described for *Z. punctata* (Álvarez et al., 2012; Lersten & Curtis, 1996; Mercado et al., 2013). Large deviations from mean values found in the longitude of leaflets and diameter of glandular trichomes could be attributed to different maturation stages.

Larrea sp. structures were found only in 20% of samples (C-I). *Larrea* samples were identified by leaf epidermal segments characterized by epidermal cells with straight anticlinal walls, cyclocytic and anomocytic stoma (20.54 ± 3.25 µm long and 27.80 ± 4.55 µm lat) (Figure 5A), abundant unicellular trichomes with acute apex (234.45 ± 145.55 µm long) with the epidermal cells arranged in groups of 4 to 6 converging toward the base of the trichome (Figure 5B), features previously described for these species by Mercado et al. (2018).

In propolis samples from Santa María, Catamarca (C-III) a nonidentified species and *Z. punctata* structures were found. The predominant nonidentified species was characterized by the presence

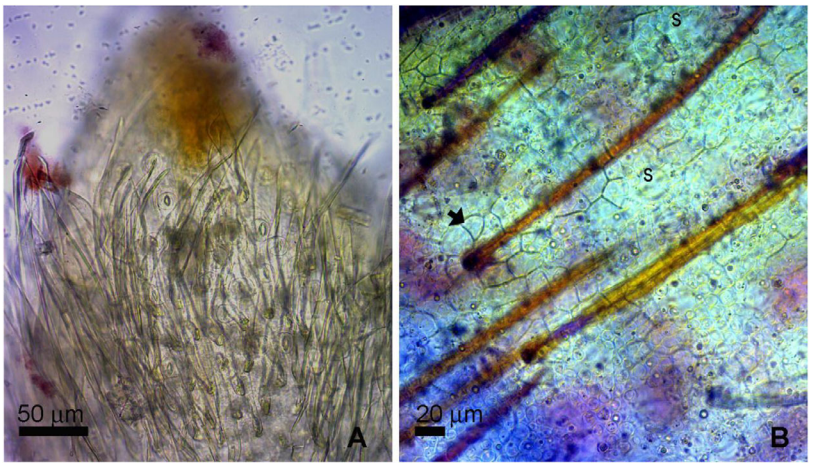

Figure 5–Botanical studies of propolis samples. Plant material identified as *Larrea* sp. (Zygophyllaceae). (A) Nonglandular unicellular trichomes with sharp apex, densely arranged on the leaf surface. (B) Epidermal cells with straight anticlinal walls, nonglandular trichome with epidermal cells arranged in groups of four to five converging toward the base of the trichome (arrow), and anomocytic stoma (s).

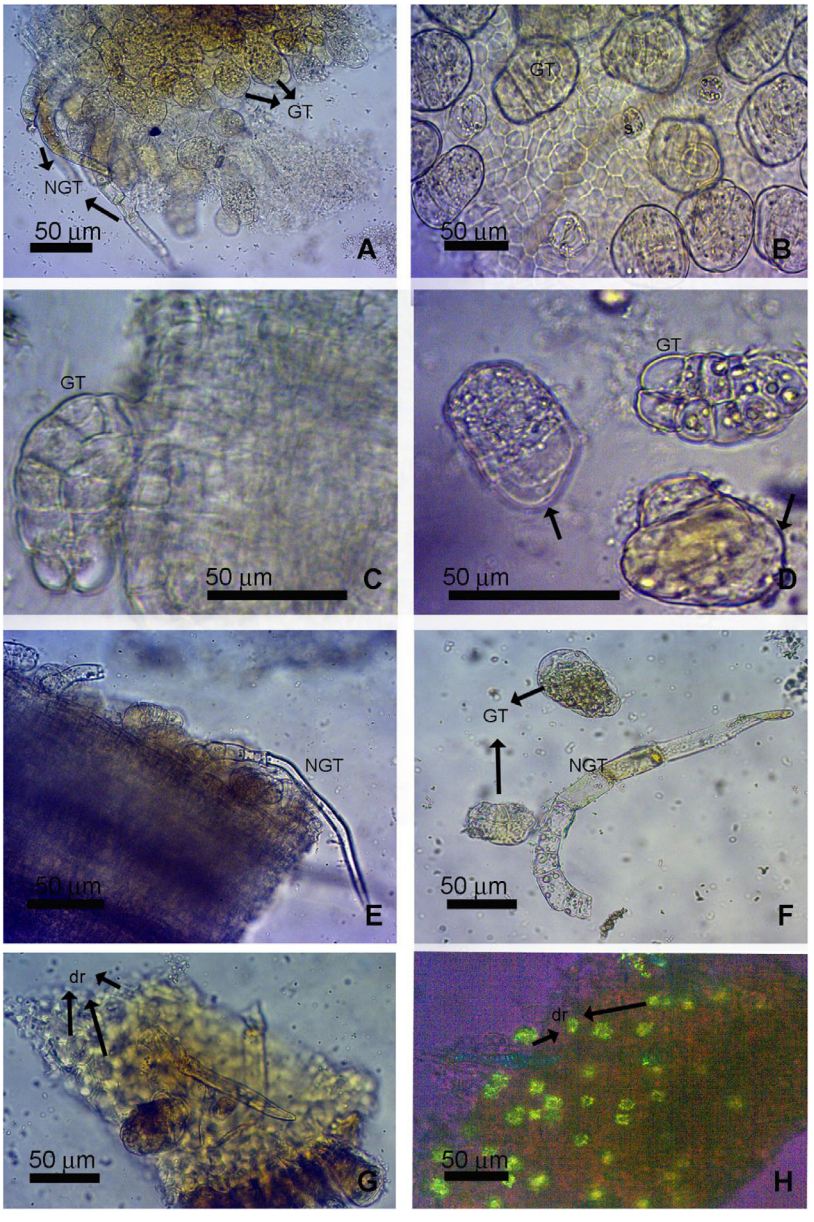

Figure 6–Botanical studies of propolis samples. Nonidentified material. (A) Foliar fragment aspect with uniseriate nonglandular trichomes (NGT), biseriate glandular trichomes (GT). (B) Epidermal fragment, epidermal cells with straight anticlinal walls, anomocytic stomata (s) and biseriate glandular trichomes (GT). (C-D) Free multicellular biseriated glandular trichomes (GT), sometimes with the cuticle slightly expanded (arrow). (E-F) Multicellular uniseriate nonglandular trichomes with an elongated apical cell. (G) Druses (dr) observed under white and (H) polarized light.

Table 2–Percentage of pollen found in samples of propolis from Catamarca.

C-I	%	C-II	%	C-III	%
Prosopis	29.6	Asteraceae	15.4	*Schinus*	19.7
Myrtaceae	12.4	*Descurainia*	14.2	*Prosopis*	18.7
Mimosa	8.7	*Gomphrena*	13.9	*Ligustrum*	12.1
Larrea	8.4	Brassicaceae	10.6	*Larrea*	11.1
Asteraceae	8	*Schinus*	10.2	Myrtaceae	9.6
Ricinus	6.2	*Prosopis*	9.9	Asteraceae	8.6
Schinus	3.6	Myrtaceae	5	Brassicaceae	7
Amaranthaceae	2.9	*Larrea*	4	*Salix*	3.5
Cactaceae	2.9	Amaranthaceae	4.4	Papaveraceae	3.5
Monocotiledonea	2.5	Monocotiledonea	4	*Plantago*	1.01
Acacia	2.2	Loranthaceae	3.7	*Gomphrena*	1.01
Descurainia	1.45	*Taraxacum*	2.2	*Celtis*	1.01
Poacea	1	*Zuccagnia*	1.5	Loranthaceae	0.5
Celtis	1	Cactaceae	0.7	*Acacia*	0.5
Ligustrum	1			*Zuccagnia*	0.5
Fumariaceae	1			*Descurainia*	0.5
Ambrosia	1			*Tecoma*	0.5
NID*	0.7			*Clematis*	0.5
Tecoma	0.7			Pinaceae	0.5
Malvaceae	0.7				
Brassicaceae	0.7				
Rubiaceae	0.36				
Polygonum	0.36				
Mutisia	0.36				
Loranthaceae	0.36				
Lamiaceae	0.36				
Ephedra	0.36				
Chorisia	0.36				

Samples from El Rincon (C-I) and Santa María (C-II, C-III). NID* Non identified

of amphistomatic leaf fragments, epidermal cells with straight anticlinal walls, anomocytic stomata (15.30 ± 4.71 µm long and 18.33 ± 5.32 µm lat), multicellular biseriated glandular trichomes with a slightly expanded cuticle (69.87 ± 21.29 µm long), multicellular uniseriated nonglandular trichomes (4 to 8 cells, 174.91 ± 49.12 µm long) with an elongated apical cell (Figure 6). In all samples, remains of pollen, spores, insect integument, and bristles were observed. Calcium oxalate druses were observed with polarized light.

According with these results, the botanical origin could mainly be ascribed to *Z. punctata*.

3.2 Melissopalynological studies

Furthermore, pollen grains in propolis samples could provide further evidence for the vegetation and the geographical origin of propolis (Guzelmeric et al., 2018). According to pollen analysis, 28 pollinic types corresponding to 29 families were identified in propolis samples from Catamarca (Table 2, Figure 7). Melissopalynological studies demonstrated that Fabaceae, Asteraceae, Zygophyllaceae, Amaranthaceae, and Brassicaceae were the only families represented by more than one pollen type. *Ambrosia* sp. (Asteraceae) and *Descurainia* sp. (Brassicaceae) were considered separately because of the abundance of their pollen grains and the accuracy for their determination.

Prosopis sp., *Larrea* sp., *Schinus* sp., Asteraceae, Myrtaceae, Brassicaceae, and Loranthacea pollen types were present in different ratios in the samples analyzed. This association indicates that the samples belong to the Monte of Sierras and Bolsones ecoregion (Cabrera, 1976; Morello et al., 2012).

The number of pollen types per sample varies between 27 and 14. C-I and C-III samples showed the highest diversity of pollen types and the highest percentages of each one (*Prosopis* sp., *Larrea* sp., *Schinus* sp.). C-II differs from the other samples with the highest amounts of Asteraceae, *Descurainia* sp. (Brassicaceae), and *Gomphrena* sp. (Table 2).

Pollen percentages ranged between 15.4 and 30% (*Prosopis* sp., *Schinus* sp., and Asteraceae) or slightly below 15.4% (*Larrea* sp., Myrtaceae, *Descurrainia* sp., and *Gomphrena* sp.) (Table 2).

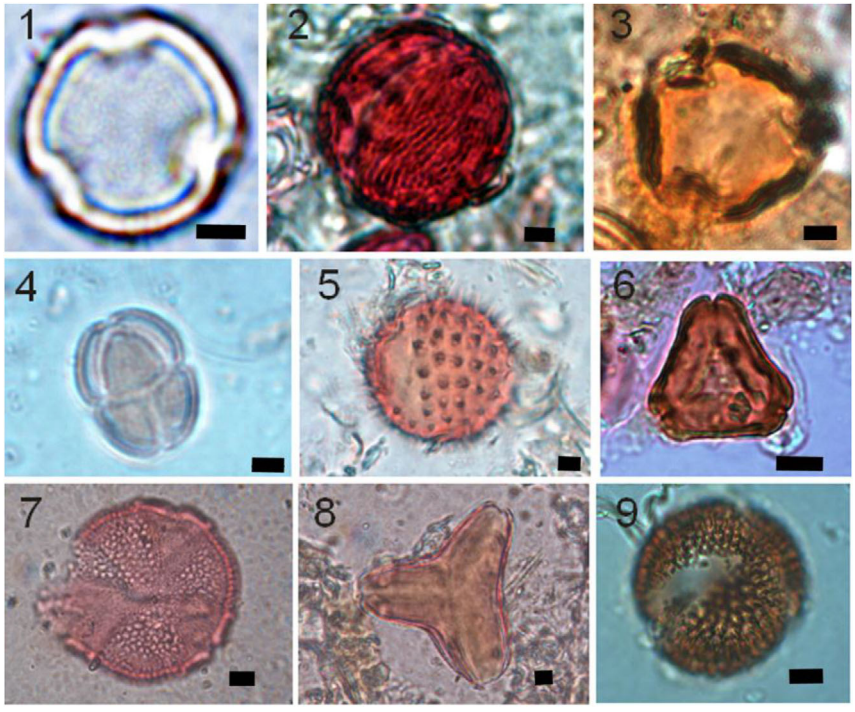

Figure 7–Main pollen types present in propolis samples. (1) *Larrea* sp. polar view. (2) *Schinus* sp. equatorial view. (3) *Prosopis* sp. polar view. (4) *Mimosa* sp. (Asteraceae) equatorial view. (5 and 6) *Myrtaceae* polar view. (7) *Zuccagnia punctata polar* view. (8) Loranthaceae polar view. (9) Brassicaceae subequatorial view. Scale 5 µm.

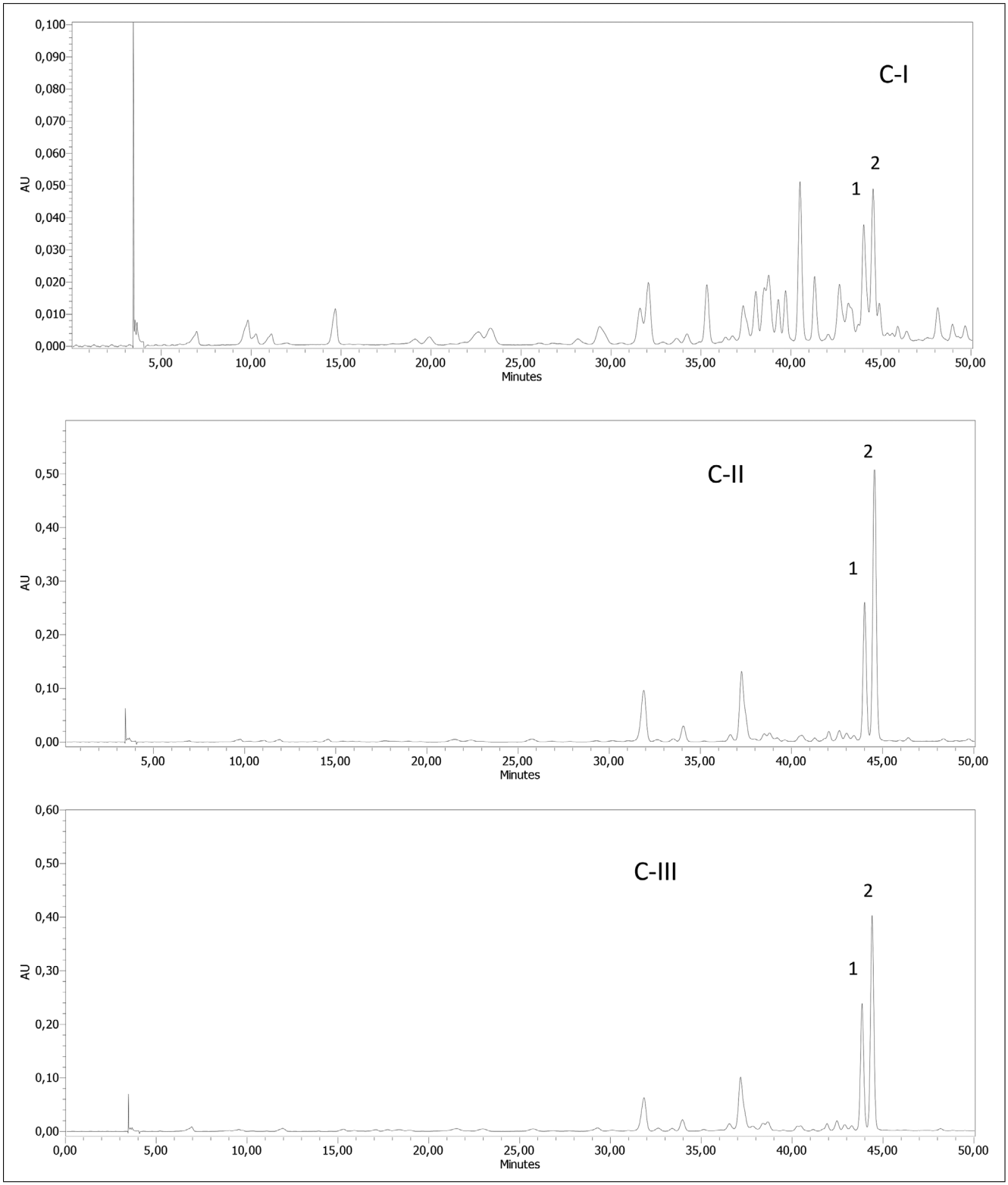

Figure 8–HPLC profiles of propolis extracts from Catamarca (330 nm). (A) Sample C-I. (B) Sample C-II. (C) Sample C-III. (1) 2′,4′-dihydroxychalcone. (2) 2′,4′-dihydroxy-3′-methoxychalcone.

Table 3–Inhibitory activity of propolis and *Z. punctata* extracts from Catamarca and isolated chalcones on enzymes related to metabolic syndrome.

Samples	IC_{50} Lipase (μg/mL)	IC_{50} α-glucosidase (μg/mL)	IC_{50} α-amylase (μg/mL)
C-I	13 ± 3[a]	11 ± 2[b]	41 ± 1[a]
C-II	25 ± 2[b]	12 ± 1[b]	40 ± 2[a]
C-III	28 ± 1[b]	7 ± 0[a]	37 ± 2[a]
Zp	23 ± 2[b]	14 ± 1[b]	42 ± 3[a]
DHC	14 ± 0[a]	8 ± 0[a]	46 ± 0[a]
DHMC	16 ± 0[a]	8 ± 1[a]	48 ± 1[a]

Samples from El Rincon (C-I) and Santa María (C-II, C-III). DHC, 2′,4′-dihydroxychalcone; DHMC, 2′,4′-dihydroxy-3′-methoxychalcone; Zp, *Zuccagnia punctata* extract; IC_{50}, inhibitory concentration of 50% enzymatic activity. Values with a common letter in the same column are not significantly different ($P \leq 0.05$).

Minor pollen types ranged between 3 and 15%, while pollen traces (T) were present in less than 3% of each sample, and were represented by native species as *Z. punctata* and *Acacia praecox* (Fabaceae), *L. divaricata* and *L. cuneifolia* (Zigophyllaceae), and *Psitacanthus* sp. (Lorantaceae). The high amounts of *Ligustrum* sp. in C-III, which is not a native species of the region, would indicate a degree of vegetation alteration probably due to human action (Table 2). The results are in agreement with previous reports that showed a large number of plant species providing nectar and pollen to honeybees; however, only a few plant species are resin sources for propolis production (Salatino & Salatino, 2017).

Our results suggested that the differences found in botanical remains compared to palynological remains in Catamarca propolis may be due to the bees selected tender parts, that is, primordial and apical buds, of resinous shrubs, such as *Z. punctata*, for the production of propolis and other plants with flowers available for honey production, although the melissopalynological analysis is not determinant of the propolis botanical origin, it provides important information about the flora surrounding the hive.

3.3 Chemical characterization

To confirm the botanical origin of propolis samples coming from the ecoregion of Monte of Sierras and Bolsones, Catamarca, a chemical characterization of PEs was carried out and compared with extracts of three jarilla species. Predominant chemical components of the propolis and *Z. punctata* extracts were (1) 2′,4′-dihydroxychalcone and (2) 2′,4′-dihydroxy-3′-methoxychalcone (Figure 8). Both compounds were previously considered as chemical markers to analyzed *Zuccagnia* type propolis samples (Salas, Mercado, Zampini, Ponessa, & Isla, 2016).

Chemical components, such as nordihydroguaiaretic acid, the major chemical compound of both *Larrea* species were not found in the PEs under study.

According with the results, the botanical origin of propolis samples from the province of Catamarca could be *Z. punctata*. Thus, we propose to name this propolis as a *Zuccagnia*-type propolis.

3.4 Inhibitory capacity of enzymes related to metabolic syndrome

The chalcone-enriched PE (C-I, C-II, and C-III) and *Z. punctata* extract showed a strong inhibitory activity for α-glucosidase and lipase, followed by α-amylase (Table 3). However, 2′,4′-dihydroxychalcone and 2′,4′-dihydroxy-3′-methoxychalcone isolated from PEs were most active, inhibiting lipase and α-glucosidase enzymes (Table 3). Other authors reported that chalcones are potent α-glucosidase, α-amylase, and lipase inhibitors (Bale et al., 2018; Cai et al., 2017; Mahapatra, Asati, & Bharti, 2015, Mahapatra, & Barthi, 2016).

Cai et al. (2017) reported that the hydroxyl chalcones showed a higher activity on α-glucosidase than methoxylated chalcones and that the position of hydroxyl groups in chalcones is another key factor for their α-glucosidase inhibitory activities, probably due to its H-bond donor effect. The inhibition of amylase might be due to the availability of the electron-accepting groups and electron-donating groups (Bale et al., 2018). The chalcones, previously considered as chemical markers in *Zuccagnia*-type propolis from Tucuman and *Z. punctata* extracts could be responsible of inhibitory activity of enzymes related to the metabolic syndrome in propolis from Catamarca, Argentina.

3.5 Antioxidant capacity

The reactive species of oxygen/nitrogen (ROS/RNS) as hydroxyl radical ($HO^{\bullet}$), hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\bullet -}$), and nitric oxide ($NO^{\bullet}$) are a health hazard, since they are able to oxidize proteins, nucleic acids, and lipids. These free radicals contribute to aging, mutagenesis, carcinogenesis, Parkinson's or Alzheimer's, inflammatory process (Kurek-Górecka et al., 2014).

In the present study, free-radical scavenging capacity of PE from Catamarca was determined. The C-II and C-III samples from Santa María were more active as $HO^{\bullet}$ scavengers (SC_{50} values of 17 and 16.5 μg/mL, respectively) than C-I sample from El Rincón (SC_{50} values of 37 μg/mL). However, C-I showed the highest power as H_2O_2 scavenger (Table 4).

On the other hand, $O_2^{\bullet -}$ plays an important role in redox cell signaling and development of pathophysiological conditions (Pistón et al., 2014). C-II and C-III samples were the most active as $O_2^{\bullet -}$ scavengers with SC_{50} values of 115 and 205 μg/mL, respectively. C-I also showed a notable capacity to scavenge nitrogen-reactive species as $NO^{\bullet}$ (Table 4).

All samples showed effective scavenging activity on ABTS with SC_{50} values between 29.5 and 33 μg/mL (Table 4). The antioxidant capacity of *Z. punctata* extracts was also demonstrated in previous reports (Carabajal, Isla, & Zampini, 2017, Carabajal, Isla, Borsarelli, & Zampini, 2020; Carabajal, Perea, Isla, & Zampini, 2020; Isla et al., 2016; Moreno et al., 2018). Other authors also evidenced scavenging activity of Argentinian PE on ABTS and DPPH radical (Nieva Moreno, Isla, Vattuone, & Sampietro, 2000, 2005; Salas et al., 2016a; Solórzano et al., 2012).

Table 4–Antioxidant activity of propolis extracts from Catamarca.

Propolis extract	SC_{50} ($ABTS^{\bullet +}$) (μg/mL)	SC_{50} ($HO^{\bullet}$) (μg/mL)	SC_{50} ($O_2^{\bullet -}$) (μg/mL)	SC_{50} (H_2O_2) (μg/mL)	SC_{50} (NO) (μg/mL)
C-I	32.2 ± 0.7[a,b]	37.0 ± 1.0[b]	290 ± 40[c]	39.00 ± 0.03[a]	50.00 ± 0.01[a]
C-II	29.5 ± 1.7[a]	17.0 ± 1.0[a]	115 ± 10[a]	58.50 ± 0.02[b]	76.40 ± 0.06[b]
C-III	33.7 ± 1.2[b]	16.5 ± 1.5[a]	205 ± 25[b]	92.00 ± 0.02[c]	104.50 ± 0.02[c]

Samples from El Rincon (C-I) and Santa María (C-II, C-III). SC_{50}, scavenging concentration of 50% of free radicals, NO, and hydrogen peroxide. Values with a common letter in the same column are not significantly different ($P \leq 0.05$).

4. CONCLUSIONS

According to the botanical and chemical analysis, *Z. punctata* is the major supplier of resins for the production of propolis from beehives located in the Monte of Sierras and Bolsones ecoregion, in Catamarca, Argentina, while the palynological profiles suggested that bees selected other plants with flowers for their food and honey production. This study is the first report that indicates that propolis samples from Argentina are effective to inhibit enzymes related to the metabolic syndrome and to free-radical scavenge activity. The results suggest that *Zuccagnia*-type propolis may be both a food supplement to control the metabolic syndrome and a potent antioxidant.

CONFLICTS OF INTEREST

The authors have stated that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: MII, ICZ.

Performed the experiments: AS, EO, MIM, FCU, MEG, JP, MAA, GP, LM, ICZ, MII.

Analyzed the data: MII, AS, MIM, ICZ, MEG, JP, GP, LM, MAA, ICZ.

Wrote the paper: MII, MIM, ICZ, AS.

Conceived and initiated the project: MII.

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